The thesis entitled "**Design, Synthesis, Conformational Analysis and Biological Studies of Peptides containing Nucleoside and Sugar Amino Acids**" consists of three chapters.

CHAPTER I: Describes the synthesis and conformational analysis of mannose-derived furanoid sugar amino acid based linear peptides.

CHAPTER II: Deals with the synthesis, conformational analysis and biological evaluation of novel cyclic cationic antimicrobial peptides containing sugar amino acids.

This chapter is divided into two parts:

Part A: Describes the synthesis and conformational studies of sugar amino acid based cyclic peptides stabilized by intramolecular H-bonding.

Part B: Describes the synthesis and biological studies of cyclic cationic antimicrobial peptides containing sugar amino acids.

CHAPTER III: Describes the synthesis and conformational studies of cyclic homooligomers of nucleoside amino acid.

CHAPTER I

Synthesis and conformational analysis of mannose-derived furanoid sugar amino acid based linear peptides

The conformational ordering of protein leading to its unique three-dimensional architecture is intriguing to chemists over the years. As the function of protein resides on its three dimensional conformation, syntheses of new non-natural scaffolds and their foldamers leading to the conformationally restricted pharmacophore could be suited for the biomedical applications. Sugar amino acid (SAA), in this context is an attractive tool in the peptidomimetic research. The functional group versatility, easy availability and sterioisomer specificity represent SAA as one of the major peptidomimetic molecular frameworks to design conformationally restrained ordered models for studying biological interactions.

We report the synthesis of sugar amino acid, methyl *N*-Boc-6-amino-2,5-anhydro-3,4-di-*O*-benzyl-6-deoxy-D-mannonate (Maa) **1** and the conformational biases imposed by the Maa residues in the linear oligomeric peptides **2-5** having repeat units of Maa(Bn_2)-Phe-Leu.

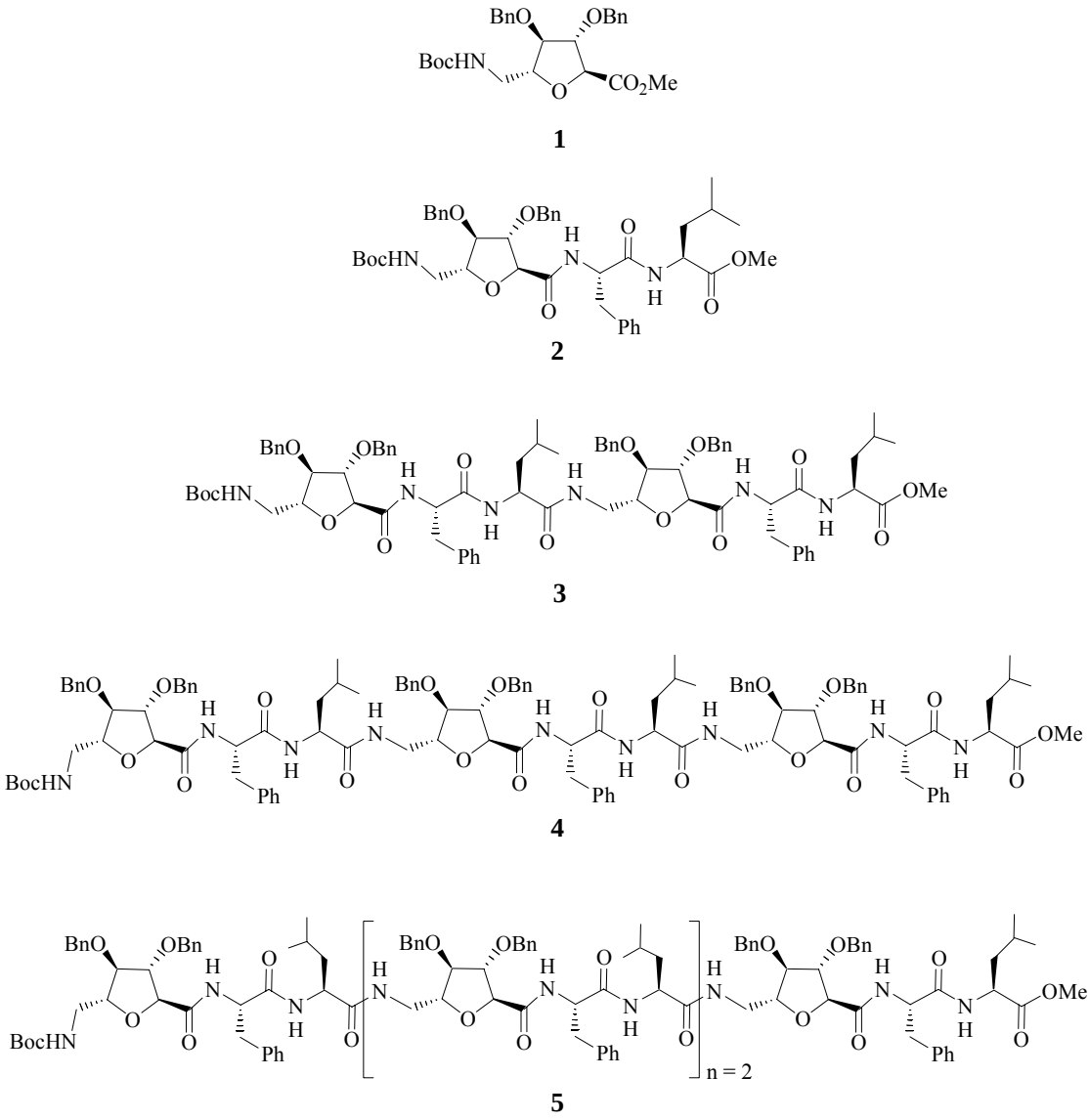
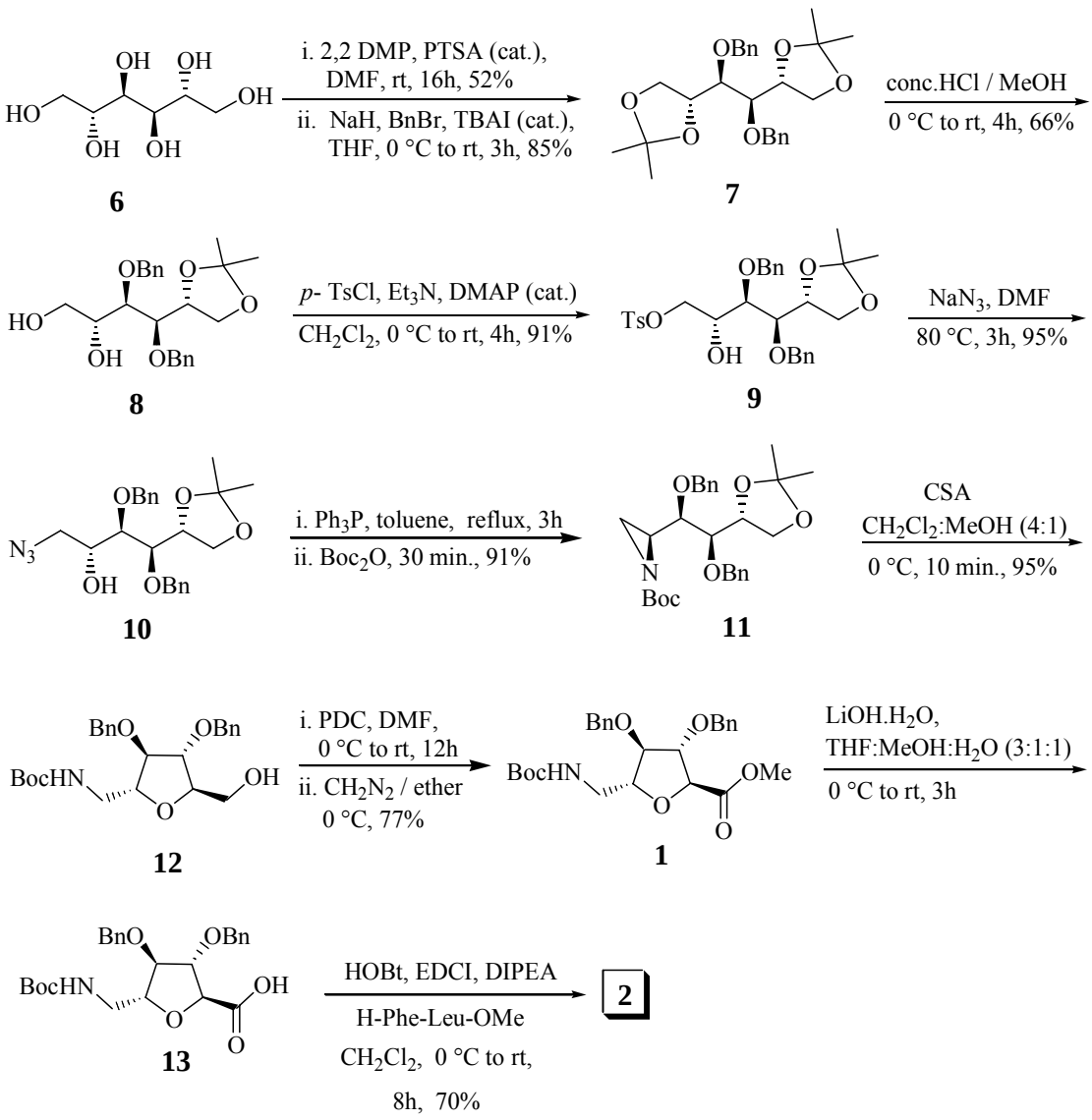


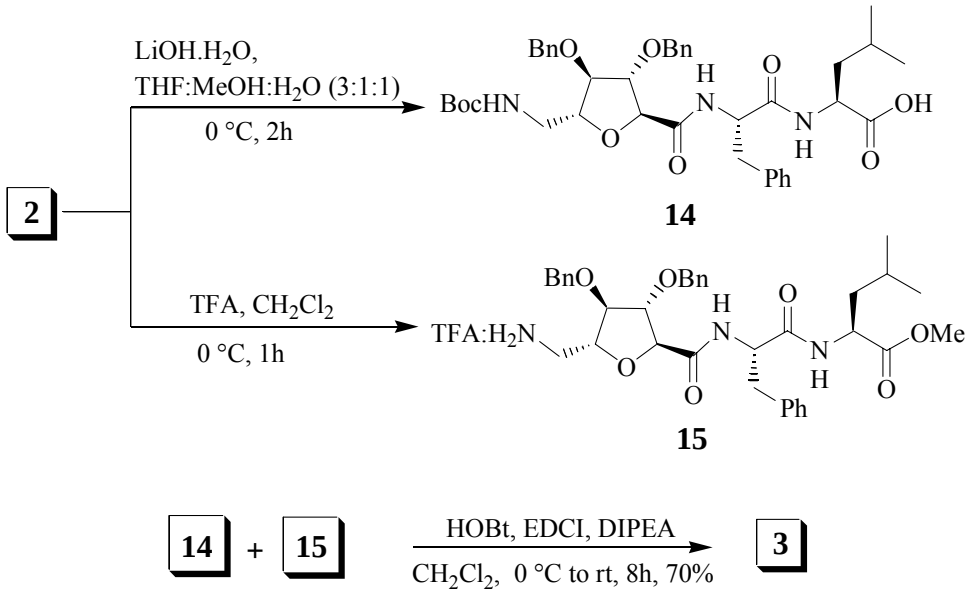
Figure 1

Our synthesis started with methyl *N*-Boc-6-amino-2,5-anhydro-3,4-di-*O*-benzyl-6-deoxy-D-mannonate (Maa) **1**, which was prepared from D-mannitol **6** following reported procedure. Scheme 1 delineates the synthesis. Saponification of **1** with $LiOH.H_2O$ in THF:MeOH:H_2O (3:1:1) gave the carboxylic acid **13**. The coupling between **13** and H-Phe-Leu-OMe under the standard peptide coupling conditions using EDCI, HOBT and DIPEA in DCM gave the tripeptide Boc-Maa(Bn_2)-Phe-Leu-OMe **2** in 70% yield (Scheme 1).



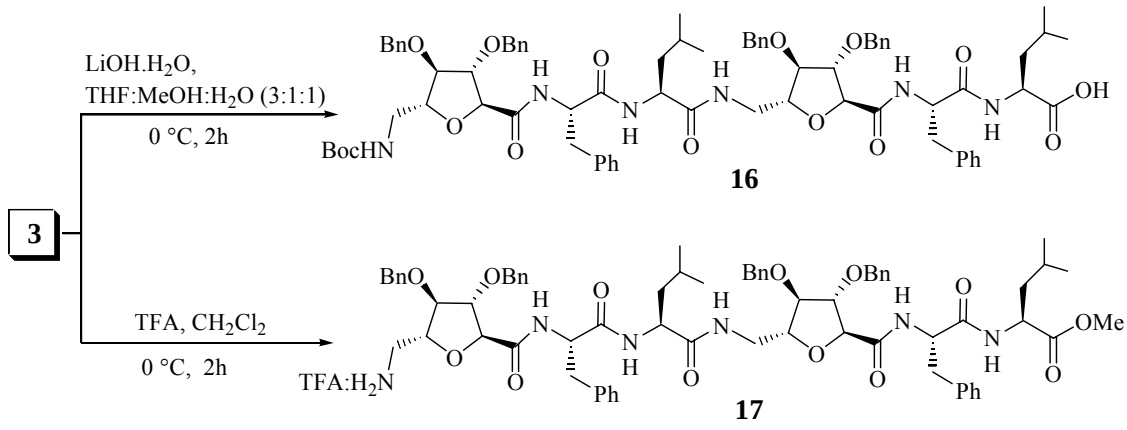
Scheme 1

Next, the tripeptide **2** was divided into two equimolar quantities. Saponification of one-half with LiOH.H_2O in THF:MeOH:H_2O (3:1:1) gave the carboxylic acid **14**. The other equivalent quantity of **2** was treated with trifluoroacetic acid in CH_2Cl_2 to give the Boc-deprotected product **15**. (Scheme 2). The coupling between **14** and **15** under standard peptide coupling conditions furnished hexapeptide **3** in 70% yield (Scheme 2).



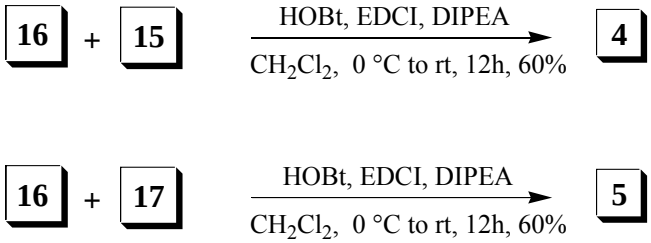
Scheme 2

Now the hexapeptide **3** was divided into three equimolar quantities. Saponification of two-half quantity separately with $LiOH.H_2O$ in $THF:MeOH:H_2O$ (3:1:1) gave the carboxylic acid **16**. The other equivalent quantity of **3** was treated with trifluoroacetic acid in CH_2Cl_2 to give the Boc-deprotected product **17** (Scheme 3).



Scheme 3

By coupling **16** with **15** and **16** with **17** under standard peptide coupling conditions we got **4** and **5**, respectively in 60% yields (Scheme 4).



Scheme 4

Conformational analysis of **2-5** was carried out by extensive NMR studies including TOCSY and ROESY experiments and molecular dynamics (MD) studies. Both **2** and **3** have a well-defined structure in $CDCl_3$ with repeating pseudo β-turns, each involving a 10-membered ring structure with intramolecular hydrogen bonds between PheNH $\rightarrow$ C=O_{i-2}. **4** and **5** aggregates to a novel antiparallel double helical structures. The 2,5-*trans* geometry of Maa leading to the significant twists in the direction of the chain propagation might have resulted the helical conformation, which is further stabilized by the extensive intermolecular hydrogen bonding that enforces the nucleation of two strands in antiparallel motif.

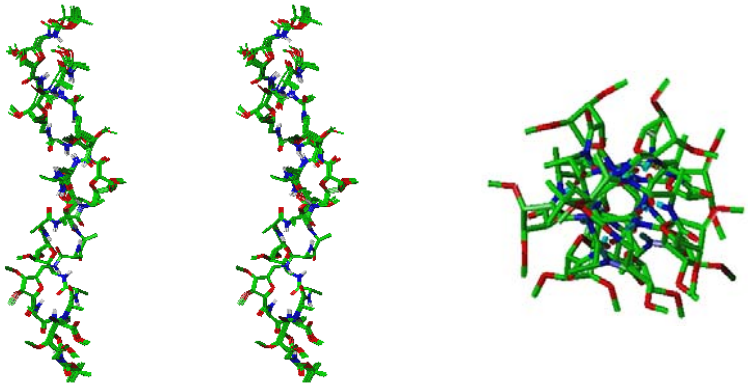


Figure 2. *Stereo-view of 10 lowest energy superimposed MD structures of* **5** *(left) and its top view (right). The benzyl groups and the amino acid side-chains are not shown for clarity.*

In conclusion, peptides containing sugar amino acid exist as antiparallel double helical fashion resembling the structural feature of Gramicidin A could be the model for new antibiotic.

CHAPTER II

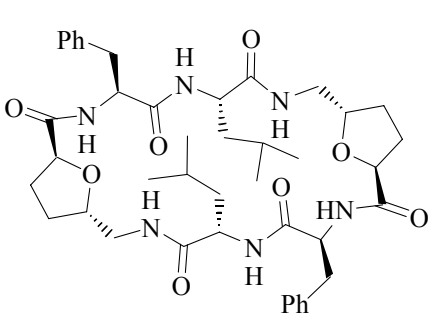
Synthesis, conformational analysis and biological evaluation of novel cyclic cationic antimicrobial peptides containing sugar amino acids

In our continuous combat against virulent pathogenic bacteria, development of new antibiotics with novel modes of action assumes great significance today. Easily curable bacterial diseases are now-a-days becoming life threatening owing to the increasing resistance of pathogens to established drugs. Cationic antimicrobial peptides (CAPs) are long considered as potential alternative antibiotics. Both linear and cyclic cationic peptides have been found as the part of innate immune response of many vertebrates including humans. These compounds exhibit a primary defense system of the host and are believed to be fighting against bacteria by disrupting their cell membrane through pore formation. These cationic antimicrobial peptides (CAPs) are having either α-helical (*e. g.* magainins, mellitin) or *β*-sheet (*e. g.* gramicidin S, tachyplesins) structure and are amphiphilic in nature. However, due to the hemolytic activity of these natural antibiotics towards the human blood cells with comparable efficiency, their uses as therapeutic agents are restricted. Medicinal chemists are thus very keen to have new CAP antibiotics either with new scaffolds or by mimicking the natural counterparts.

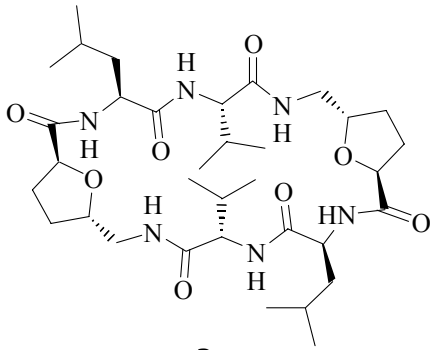
Sugar amino acids (SAAs) by virtue of its versatile functionalities could be useful scaffolds for peptide antibiotic research. We intended to synthesize cyclic cationic antimicrobial peptides, whose cyclic frame will be rigidified by intramolecular H-bonding and the peptides will be amphiphilic, i.e., there will be segregation of hydrophobic and hydrophilic part onto separate surfaces.

Part A: Synthesis and conformational studies of sugar amino acid based cyclic peptides stabilized by intramolecular H-bonding.

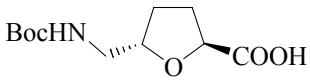
In this part we did approach to design and synthesize cyclic compounds that could exhibit secondary structure through intramolecular H-bonding. Herein we describe the synthesis of cyclic peptides **1-2**, containing sugar amino acid **3** (Figure 1). These cyclic peptides were studied in detail for their conformational preferences.



1



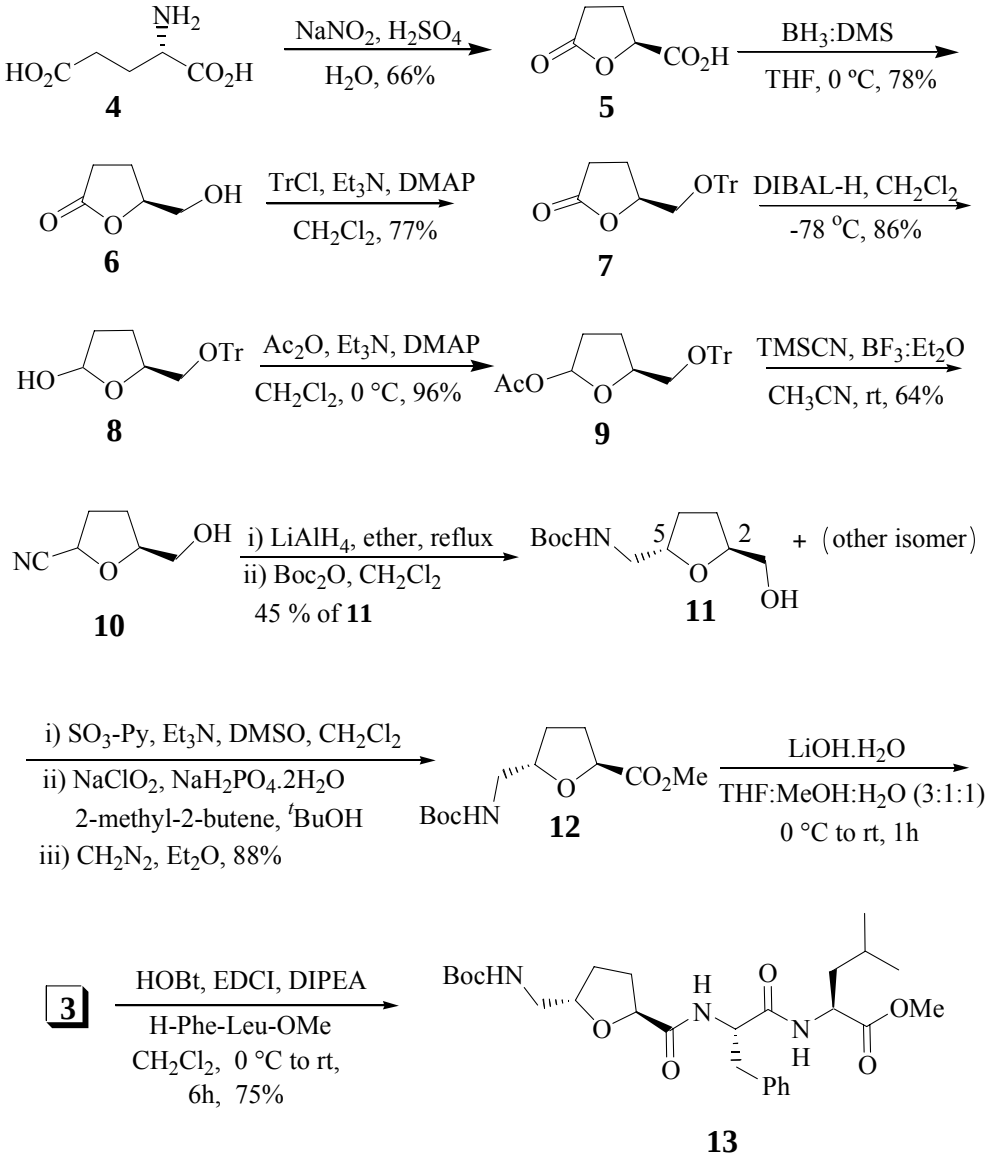
2



3

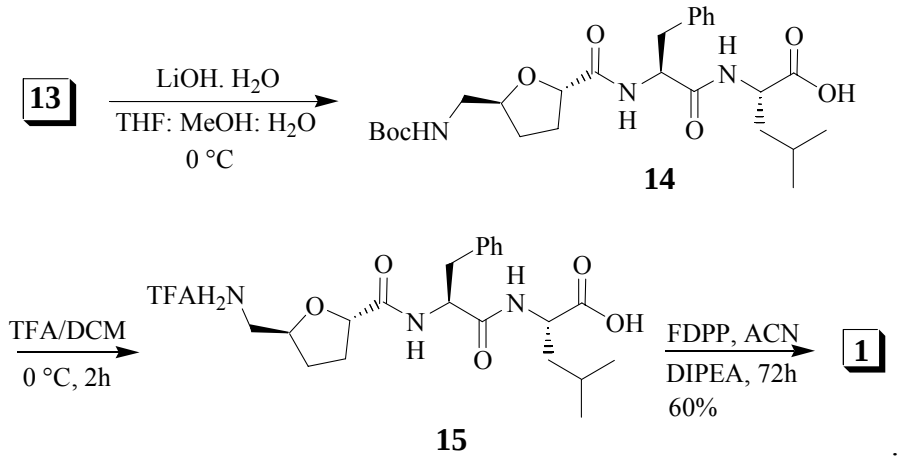
Figure 1

Our synthesis started from (2*S*)-5-oxotetrahydro-2-furancarboxylic acid **5**, which was prepared from L-glutamic acid **4**, following the reported procedure (Scheme 1). The compound **5** was reduced to alcohol **6** in 78% yield, upon treatment with borane-dimethyl sulfide in anhydrous THF at 0 ºC to room temperature. Protection of the primary alcohol by using TrCl and Et_3N with catalytic DMAP in DCM furnished **7** in 77% yield. Lactone was selectively reduced to inseparable anomeric mixture of lactol **8** using DIBAL-H in 86% yield. Acetylation of anomeric alcohol using Ac_2O, Et_3N gave **9** in 96% yield. **9** was treated with trimethylsilyl cyanide in the presence of BF_3:Et_2O to give a diastereomeric mixture of the glycosyl cyanides **10**, in 64% yield. Treatment of compound **10** with $LiAlH_4$ in refluxing condition in THF resulted the primary amine, which was *in situ* protected with Boc_2O to furnish **11** (α:β, 45:30) in 75% yield. Finally, two steps oxidation of primary alcohol of **11** followed by treatment with CH_2N_2 resulted in the formation of **12** (88% yield). Saponification of **12** with $LiOH.H_2O$ in THF:MeOH:H_2O (3:1:1) gave the sugar amino acid **3**. The coupling between **3** and H-Phe-Leu-OMe under the standard peptide coupling conditions using EDCI, HOBT and DIPEA in DCM gave the tripeptide Boc-SAA-Phe-Leu-OMe **13** in 75% yield (Scheme 1).



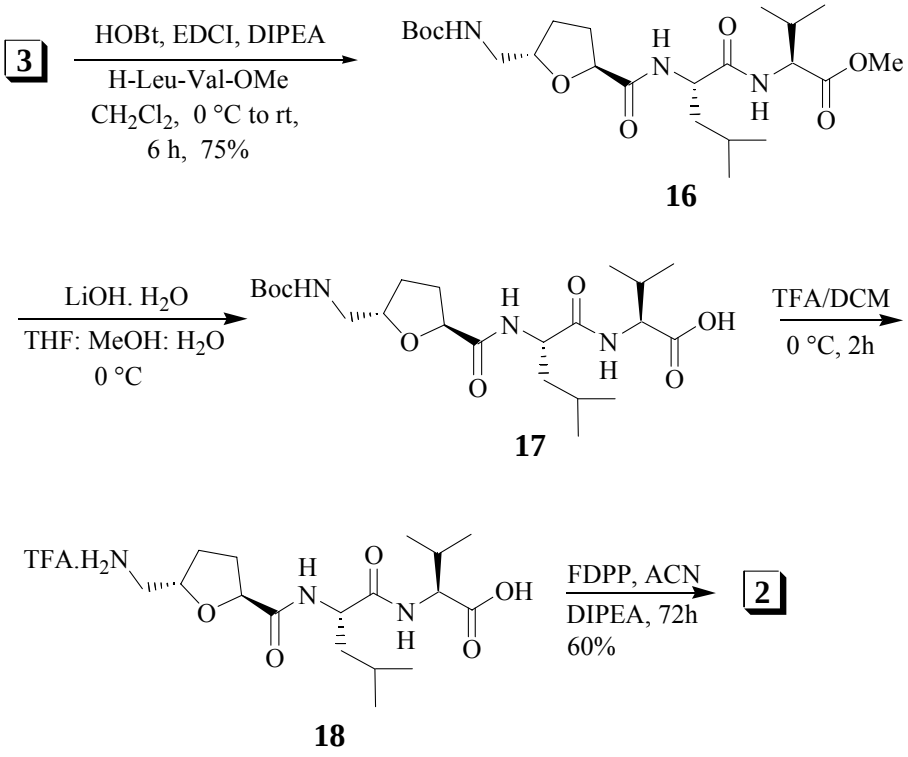
Scheme 1

Saponification of compound **13** was performed with $LiOH.H_2O$ in $THF:MeOH:H_2O$ (3:1:1) to furnish the acid **14**. Next, the crude acid **14** was treated with TFA in CH_2Cl_2, to give the TFA-salt **15** (Scheme 2). When the TFA-salt **15** was treated with pentaflouro diphenyl phosphinate (FDPP) and DIPEA in dry acetonitrile (10^{-3} M) at 0 °C to room temperature, it underwent cyclodimerization reaction to give **1**, the only isolated product in 60% yield (Scheme 2)



Scheme 2

To reproduce this cyclodimerisation reaction, another analogue **2** was also prepared. For that, **3** was coupled with H-Leu-Val-OMe to get tripeptide **16**. Following the above-mentioned procedure **16** was cyclodimerised to furnish **2** (scheme 3).



Scheme 3

The NMR spectra of the cyclic products displayed symmetrical structures. Detailed NMR studies in $CDCl_3$ and subsequent constrained MD simulations revealed that **1** and **2** displayed well-defined distorted "*β*-*β* corner" structures where both PheN*H* and SAANH in **1** and LeuN*H* and SAANH in **2** are H-bonded and each 24 membered macrocyclic ring is stabilized by four intramolecular H-bonds (Figure 2).

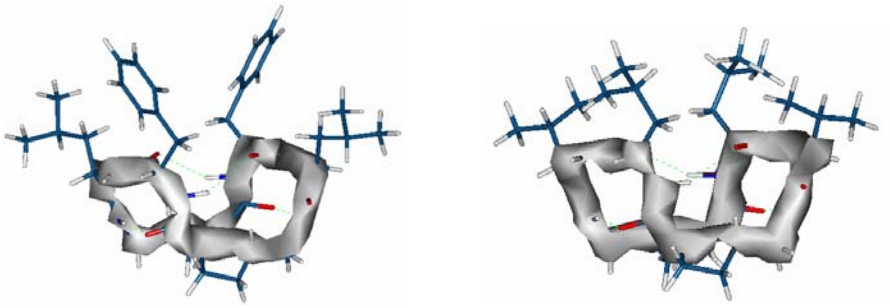
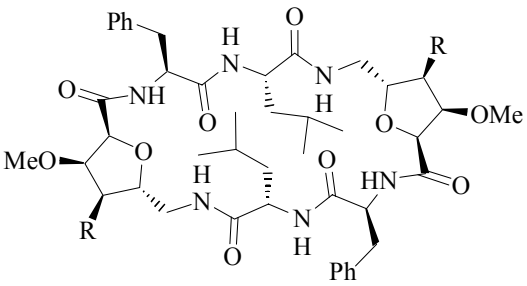


Figure 2. *Lowest energy structure of **1** (left) and **2** (right) obtained from MD calculations.*

Part B: Synthesis and biological studies of cyclic cationic antimicrobial peptides containing sugar amino acids.

In previous part of this chapter we synthesized well-defined intramolecularly hydrogen-bonded furanoid sugar amino acid based rigid cyclic peptides as basic frame. Our next target was to impose amphiphilic nature to those secondary structured cyclic peptides by distributing the hydrophobic and hydrophilic residues onto separate surfaces, which is pre-requisite for peptides to act as CAPs. Knowing its conformational features we anticipated that tuning in the sugar amino acids with suitable functionalities could lead to the generation of novel amphiphilic structures to our cyclic compounds.

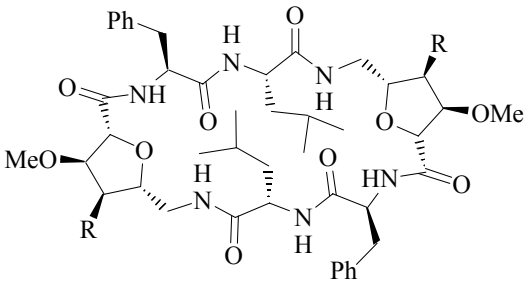
We report here synthesis, conformational analysis of a set of cyclic peptides (**19-21**) that are potentially active against bacteria.



19a, R = NH_3^+

20a, R = $NHCOCH_2CH_2NH_3^+$

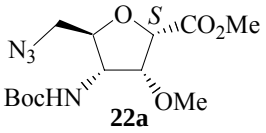
21a, R = $NHCOCH(NH_3^+)(CH_2)_4NH_3^+$



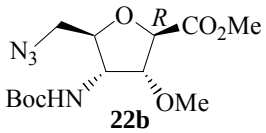
19b, R = NH_3^+

20b, R = $NHCOCH_2CH_2NH_3^+$

21b, R = $NHCOCH(NH_3^+)(CH_2)_4NH_3^+$



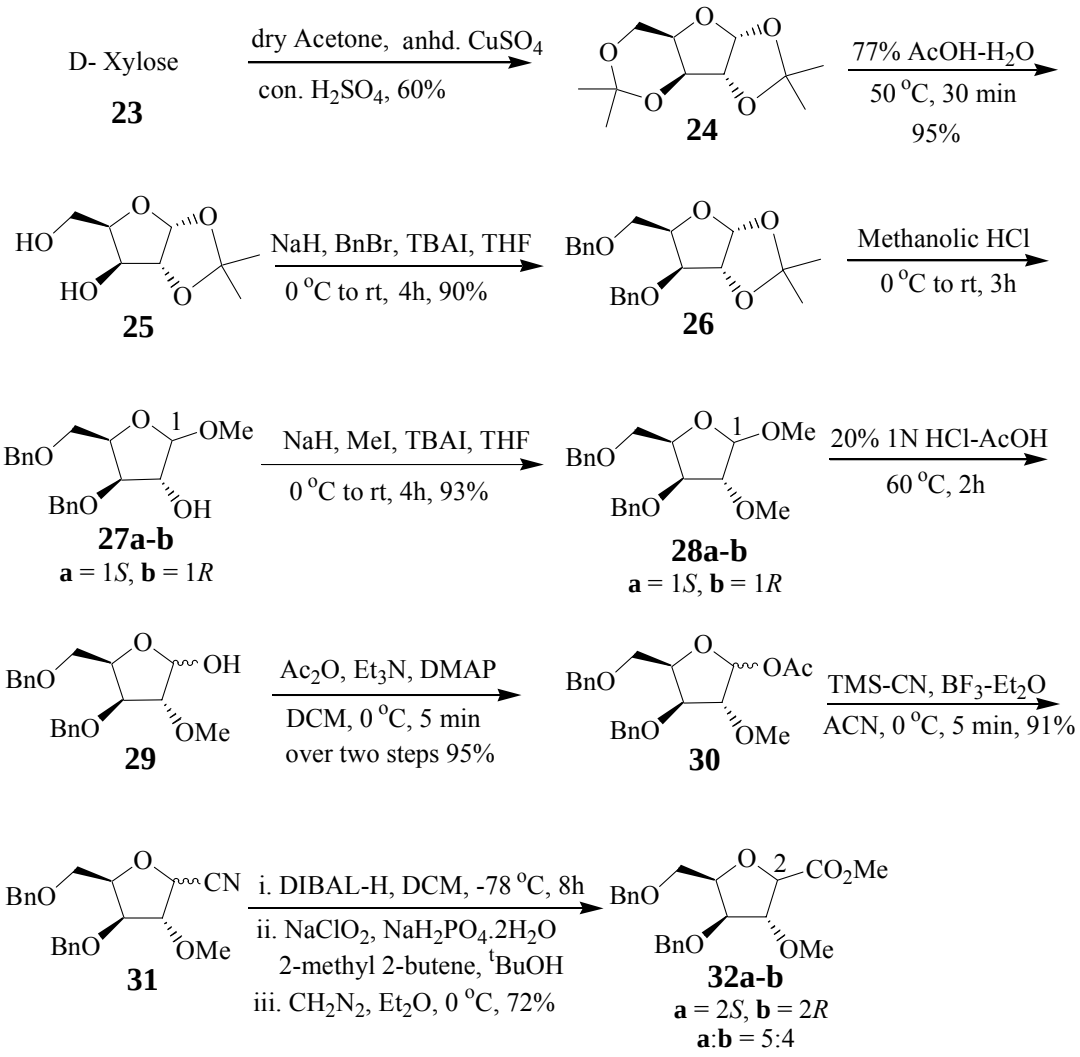
22a



22b

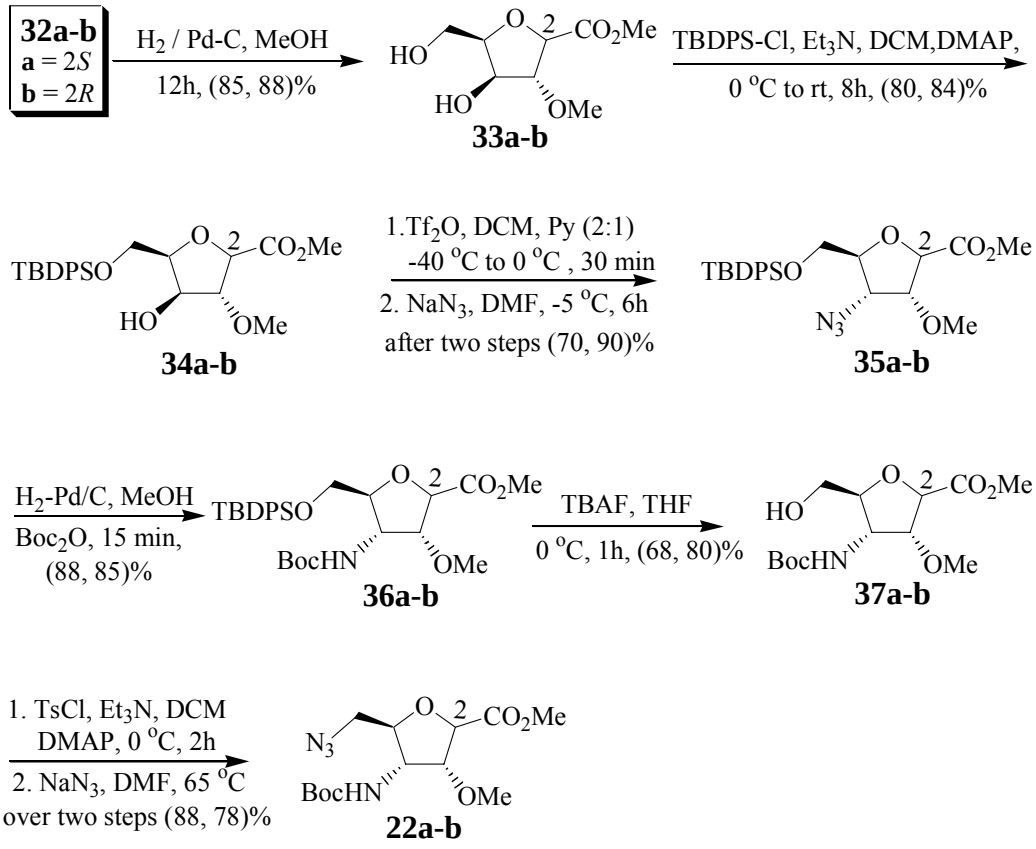
Figure 3

The functionally tuned building blocks **22a** and **22b** were used to synthesize the cyclic compounds. Introduction of an extra amino group in C-4 position of SAA ring was our interest from synthetic point of view. We were also interested to devise a common strategy to synthesize these two diasteriomeric SAAs from the same starting material. Our synthesis of the building block **22** started from **26,** which was prepared from D-xylose (**23**) following literature procedure (Scheme 4). The 1,2-*O*-isopropylidene group of **26** was methanolyzed upon treatment with dry HCl in MeOH to yield both the anomers **27a-b** of methyl-3,5-di-*O*-benzyl-D-xylofuranoside in a ratio of 2:3 (α:β), respectively. The C-2 hydroxyl was etherified using MeI, NaH and catalytic amount of TBAI. Next the compound was treated with 20% 1NHCl-AcOH and heated at 60 °C for 2h. The hemiacetal was acetylated to **30** without any further purification using Ac_2O/Et_3N. The inseparable anomers (**30**) were treated with TMS-CN in presence of BF_3-Et_2O to furnish **31** as anomeric mixture. Conversion of CN to aldehyde using DIBAL-H, oxidation and treatment with CH_2N_2 resulted **32a-b** (5:4) in 72% yield (Scheme 4). The anomers could easily be separated through silica gel column chromatography. The next reactions of these isomers were performed separately.



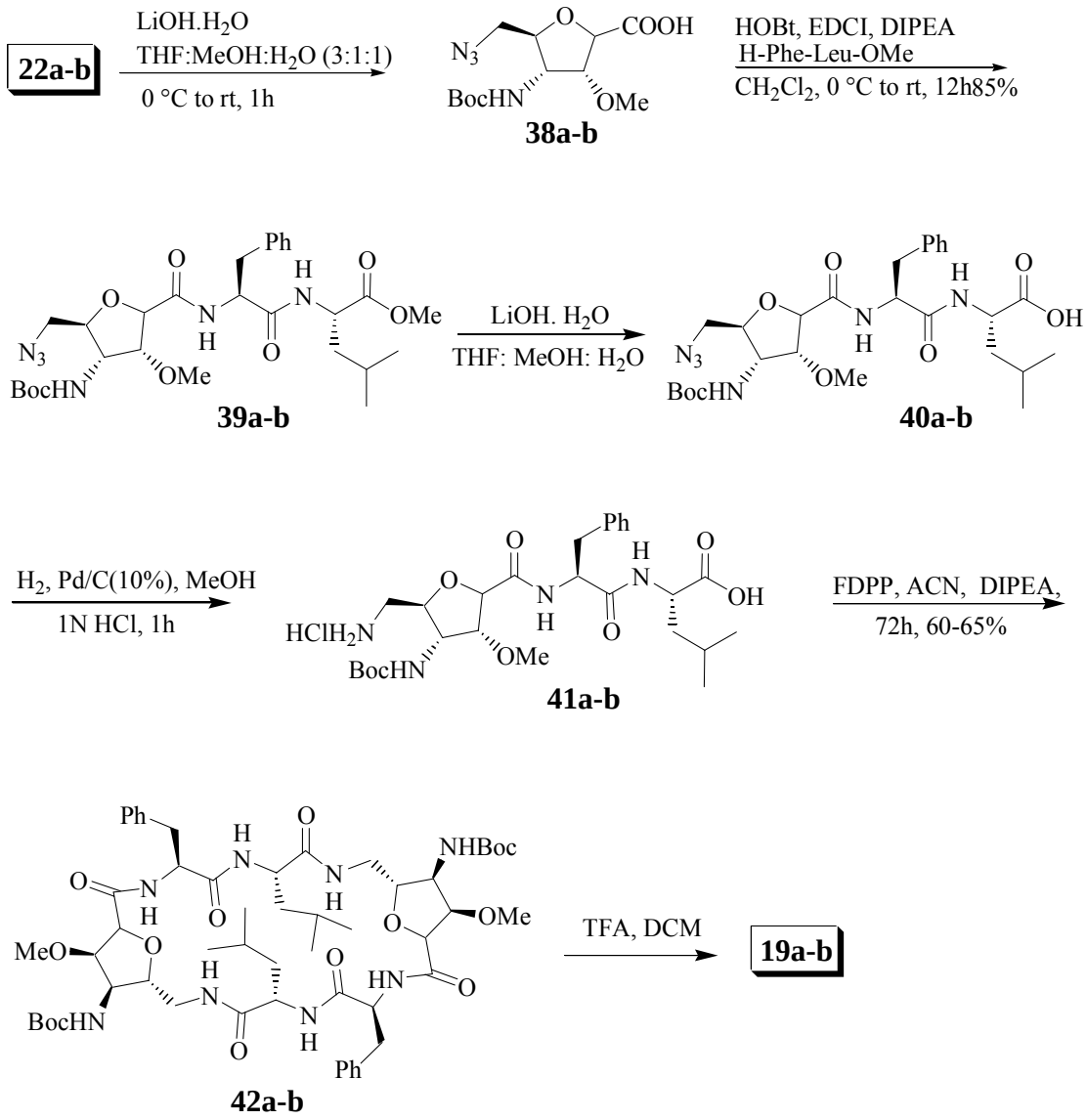
Scheme 4

Complete debenzylation followed by selective TBDPS protection to primary hydroxyl yielded **34a-b**. Our next target was to introduce azido group to C-4 center. C-4 hydroxyl was reacted with Tf_2O. The resulting triflate, after flash chromatography was treated with 6 eqv. of NaN_3 in dry DMF at -5 °C to furnish γ azido ester **35a-b**. The azido group was hydrogenated in the subsequent step and the resulting amino group was protected *in situ* by Boc_2O to yield **36a-b**. Little elimination was observed in α-anomer that was inseparable from **35a**. However, the eliminated product could be separated in 8% yield in its saturated form in the next step. Primary silyl deprotection using TBAF afforded **37a-b**. Tosylation of the primary hydroxyl group and upon heating the tosylated intermediate with NaN_3 in DMF at 65 °C yielded SAAs **22a-b** (Scheme 5).



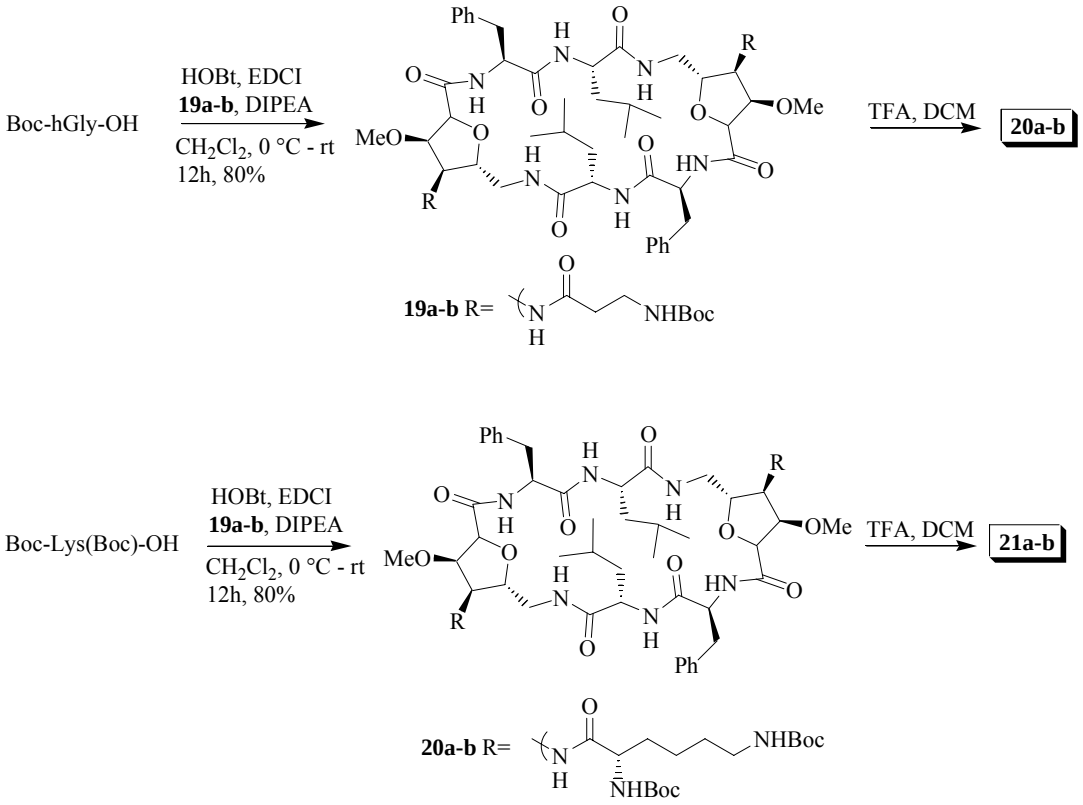
Scheme 5

Synthesis of cyclic framework of the target molecules is described in Scheme 6. Saponification of SAA **22a-b** followed by coupling with H-Phe-Leu-OMe using EDCI and HOBt afforded **39a-b** in 85% yield. Stepwise saponification of **39a-b** followed by hydrogenation of azido to amine yielded the crude tripeptide which on the subsequent step were cyclodimerised using FDPP in CH_3CN under dilute condition (2×10^{-3} M) to furnish **42a-b** in 60-65% yield. Removal of Boc using TFA in DCM afforded **19a-b** in quantitative yield (Scheme 6).



Scheme 6

19a-b were separately coupled with Boc-hGly-OH and Boc-Lys(Boc)-OH to furnish **43a-b** and **44a-b**, respectively, following the procedure stated earlier. Finally global Boc-deprotection of each compound furnished **20a-b** and **21a-b** in quantitative yields (Scheme 7).



Scheme 7

The compounds **19a-b**, **20a-b** and **21a-b** were bacteriolytic. The antibacterial activity of the compounds was examined against *E. coli* and *S. aureus*. The compounds with (2*S*) configuration in SAA were more active against *E. coli* and *S. aureus* as compared to the compounds where the SAA was (2*R*). The presence of $-NH_2$ groups and hydrophobicity provided by the Leu and Phe side chains, imparted cell-surface disrupting properties as observed for cationic antibacterial peptides. Antibacterial activities were at lower concentration compared to hemolytic activity.

In conclusion, novel cyclic cationic peptides containing sugar amino acids were synthesized and they were found to act against bacteria. The higher antimicrobial activity and lower hemolytic activity could make them attractive tools for the rational design of antibiotic.

CHAPTER III

Synthesis and conformational studies of cyclic homooligomers of nucleoside amino acid.

Cyclisation of linear biopolymers is widely used to constrain their conformational degrees of freedom and induce desirable structural biases permitting enhanced receptor selectivity and binding affinity with additional properties like decreased susceptibility to degradation in biological systems. Cyclic DNAs and RNAs, for example, have been studied extensively for their unusual chemical and biological activities. However, synthesis of such cyclic DNAs remains a challenging task, thereby limiting exploratory studies, especially in discovering potential leads for drug discovery. It was envisaged that the replacement of the phosphodiester linkages with amide bonds would not only facilitate the assembly of such substrates using standard solid- or solution-phase peptide synthesis methods, but would also help to enhance their stability towards nucleases.

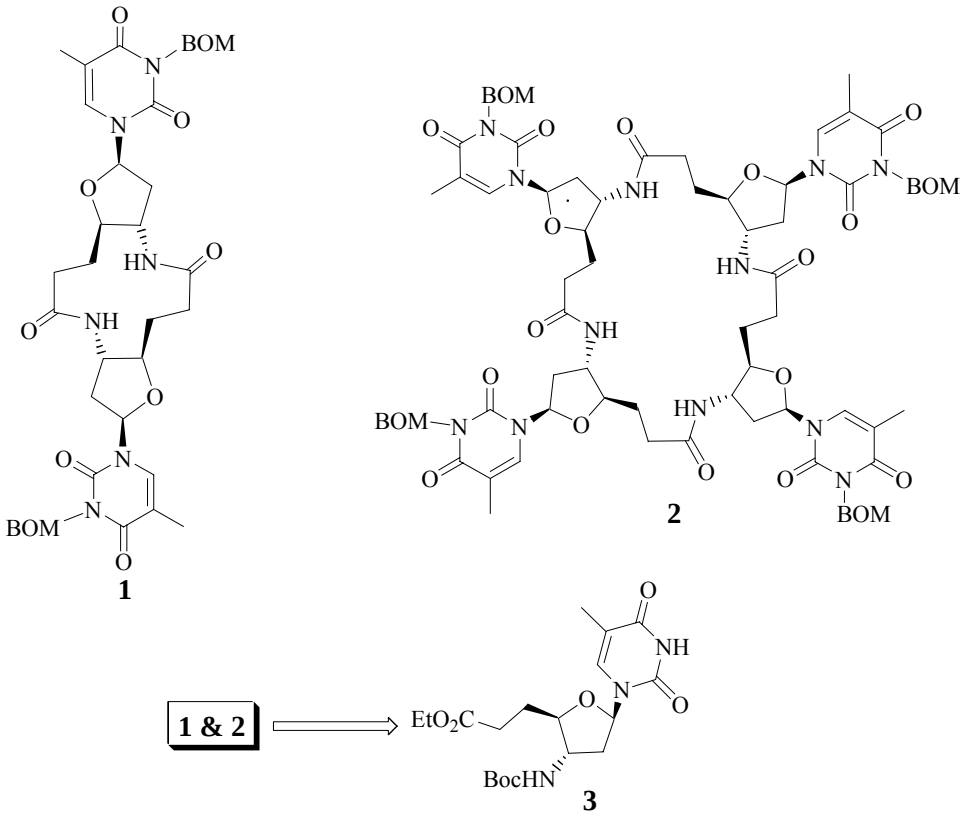
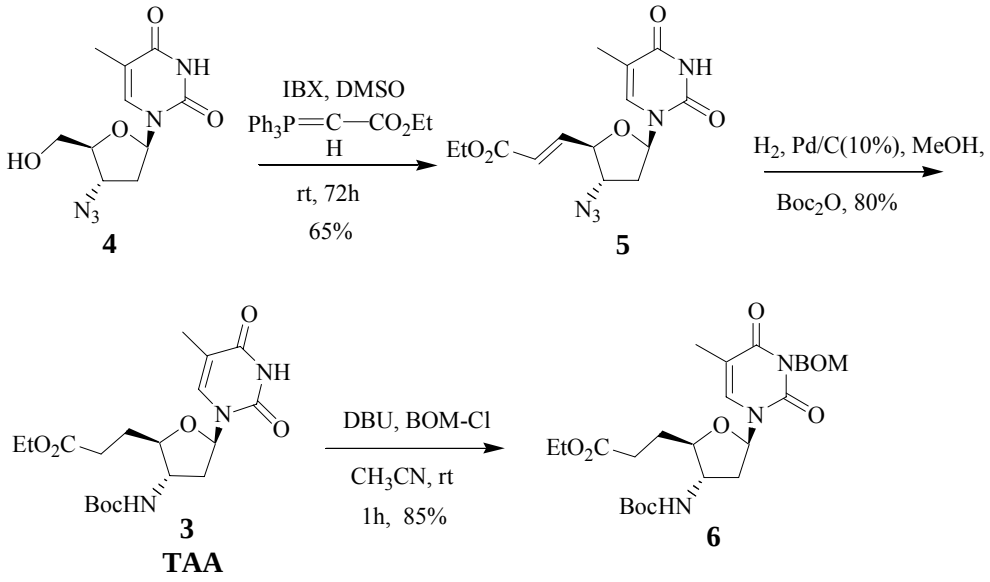


Figure 1

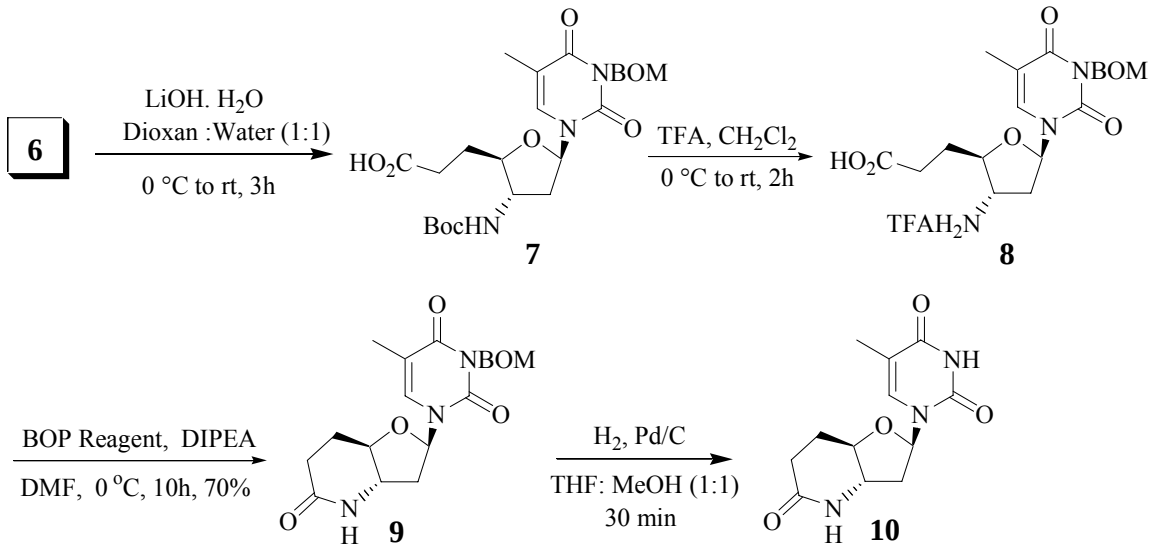
Amide-linked oligonucleotides have been studied extensively for potential therapeutic applications involving antisense strategy. However, their cyclic versions have remained largely unexplored. Herein, we describe the synthesis of amide-linked cyclic homooligonucleotides **1** and **2**, of monomeric building block **3**, (Figure 1) a thymidine-based nucleoside amino acid (Taa). These cyclic homooligomers **1** and **2** were studied in detail for their conformational preferences and their biological activity.

Our synthesis was started from AZT (**4**), which was prepared from thymidine following reported procedure. The oxidation of AZT and Wittig olefination of the resulting aldehyde was accomplished in a one-pot process using iodoxybenzoic acid (IBX) in the presence of stabilized ylide, $Ph_3P{=}CHCO_2Et$ in DMSO to give exclusively *trans* α,β-unsaturated ester compound **5** in 65% yield (Scheme 1). Then both the olefin and azide were reduced under Pd-catalyzed hydrogenation condition using Pd/C in methanol and the resulting amino compound was protected with Boc *in situ* to get the protected nucleoside δ-amino acid TAA (Thymidine Amino Acid) **3**. The amidine N*H* proton was then protected with benzyloxy methyl chloride (BOM-Cl) using DBU as base to get **6** in 85% yield (Scheme 1).



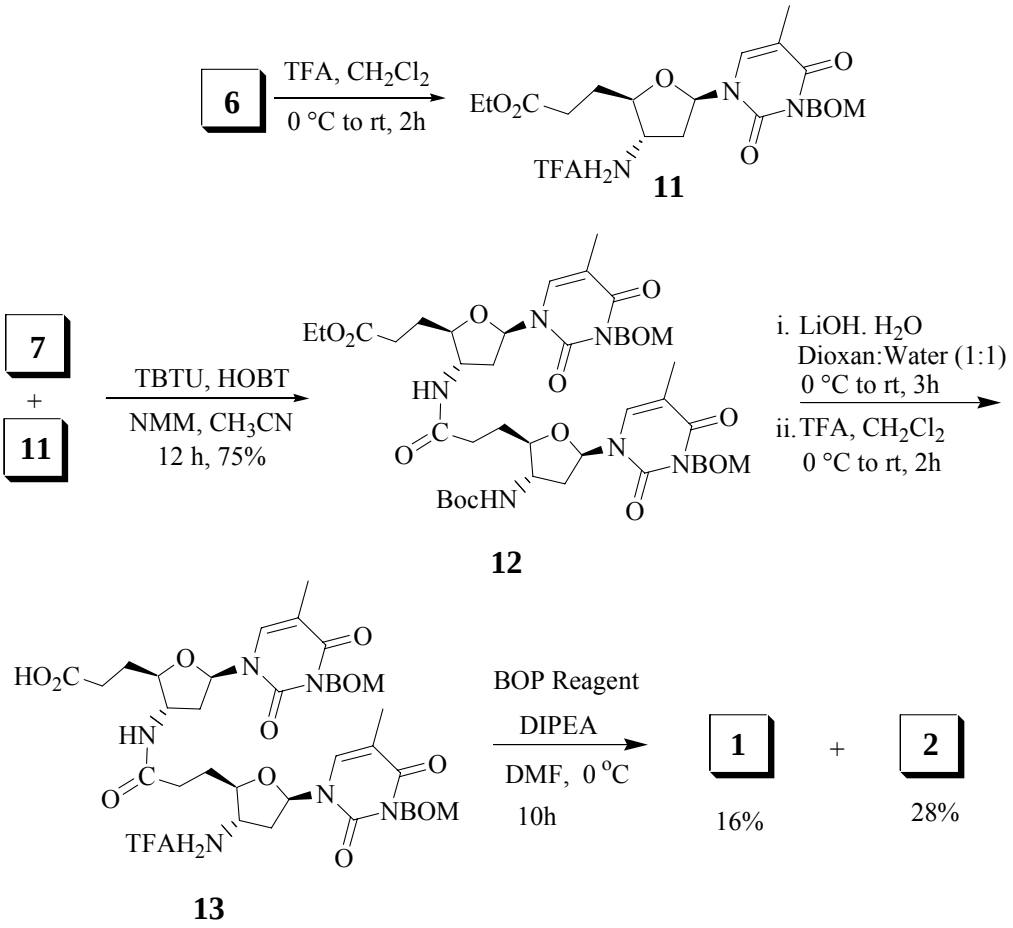
Scheme 1

The method that we planned to use for the synthesis of macrocyclic peptides is based on a reaction, which was expected to convert the nucleoside amino acid monomers directly into their cyclic homooligomers. Accordingly, saponification of compound **6** was performed with $LiOH.H_2O$ in Dioxan:H_2O (1:1) to furnish **7**. Next, the crude acid of **7** was treated with TFA in CH_2Cl_2, which yielded the TFA-salt **8** (Scheme 2). When **8** was treated with BOP reagent, **9** was the only isolated product. There was no trace of cyclooligomerized product. Hydrogenation of **9** yielded **10,** which proved the formation of bicyclic lactam.



Scheme 2

Then we changed our strategy to get requisite cyclic homooligomers by preparing linear dimer. The linear dimer was prepared by conventional solution phase peptide synthesis methods, using TBTU and HOBt as coupling agents. Thus, **6** was treated with TFA in CH_2Cl_2 yielded the Boc-deprotected compound **11**. The coupling between **7** and **11** was performed to furnish the linear dimer **12** in 75% yield (Scheme 3). Stepwise saponification of **12** followed by Boc deprotection yielded **13** which on the subsequent step when kept for cyclisation reaction using BOP reagent under dilute condition, **1** and **2** were isolated in 16% and 28% yield, respectively (Scheme 3).



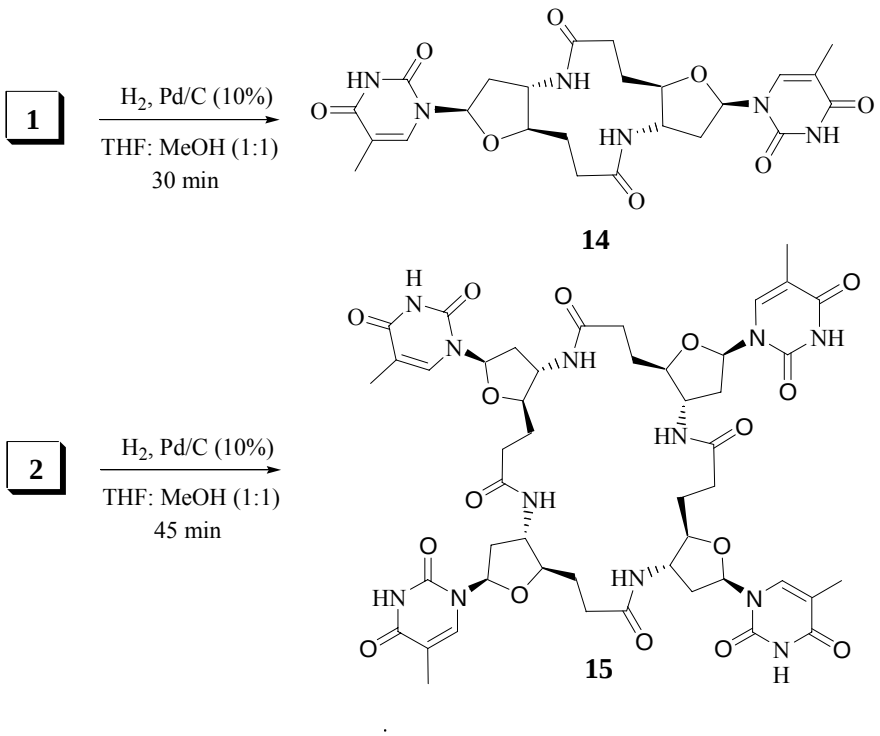
Scheme 3

The conformational analysis of **1** and **2** were carried out by NMR spectroscopy in $CDCl_3$ and DMSO-d_6, respectively. The presence of only one set of peaks in **1** and **2** is consistent with a two and four fold symmetry respectively. The resulting structure had the NH and CO pointing approximately perpendicular to the plane of the macrocyclic ring with CO on the same side as the base (Figure 2).

Figure 2. *Stereo view of the 20 superimposed energy-minimized structures of **1***

None of these cyclic compounds showed any significant activity against bacteria. Finally, the BOM-protections of both the cyclic homooligomers **1** and **2**

were removed by Pd-catalyzed hydrogenation to furnish **14** and **15** (scheme 4). The conformational analysis of **14** and **15** could not be carried out due to line-broadening and overlapping signals both in $CDCl_3$ and DMSO-d_6, making it very difficult to derive the spectral parameters.



Scheme 4

In conclusion, cyclic homooligomers of nucleoside amino acid constitute a new class of novel molecular entities that display interesting 3-D structures, reminiscent of the structures of peptide nanotubes. The well-defined structures of these macrocyclic peptides will be useful to carry out investigations on many interesting molecular recognition processes.